

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 27 MAY 2005

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Applicant s or agent s file reference 300420RU	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/RU 2003/000525	International filing date (day/month/year) 26 November 2003 (26.11.2003)	Priority date (day/month/year) 26 December 2002 (26.12.2002)
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Applicant ZAKRYTOE AKTSIONERNOE OBSHESTVO "EVROGEN" et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This Report consists of a total of 4 sheets, including this cover sheet.

- ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under PCT).

These annexes consist of a total of 3 sheet

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand: 24 June 2004 (24.06.2004)	Date of completion of this report: 31 March 2005 (31.03.2005)
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# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.  
PCT/RU 2003/000525

## I. Basis of the report

### 1. With regard to the elements of the international application:\*

☐ the international application as originally filed

☒ the description:

pages 1-24, as originally filed  
pages, filed with the demand  
pages, filed with the letter of

☒ the claims:

pages, as originally filed  
pages, as amended (together with statement) under Article 19  
pages, filed with the demand  
pages 25-27, filed with the letter 21.12.2004

☒ the drawings:

pages 1/5-5/5, as originally filed  
pages, filed with the demand  
pages, filed with the letter of

☒ the sequence listing part of the description:

pages 1-21, as originally filed  
pages, filed with the demand  
pages, filed with the letter of

### 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1.(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

### 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.  
☒ filed together with international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

### 4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages \_\_\_\_\_  
☐ the claims, Nos. \_\_\_\_\_  
☐ the drawings, sheets/fig. \_\_\_\_\_

### 5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

## 1. Statement

Novelty (N)	Claims	1-24	YES
	Claims		NO
Inventive Step (IS)	Claims	1-24	YES
	Claims		NO
Industrial Applicability (IA)	Claims	1-24	YES
	Claims		NO

## 2. Citations and explanation:

The examination report is established for amended claims in view of the following documents:

D1 – AAN<sub>2</sub>52735  
D2 – JP 10-234382;  
D3 – US 6486382;  
D4 – WO 9937142  
D5 – US 5976796  
D6 – US 6232107

D1 discloses a primary structure of green fluorescent protein having an amino acid sequence, which is about 25% identical to SEQ ID NO:2 characterizing the nucleic acid according to claim 1.

D2 discloses information about a nucleic acid sequence encoding green fluorescent protein, a vector comprising thereof, host cells capable of synthesizing the indicated fluorescent protein. D2 also provides possibility for the use of the obtained protein as a labeling agent for detecting the protein localization in live cells, as a reporter for the analyses of promoters, etc.

D3 discloses a plant comprising a nucleic acid molecule encoding green fluorescent protein included in expression vector.

D4 discloses a method for producing a transgenic non-human mammal carrying a transgene encoding an immunoinducible autofluorescent protein, such as Green Fluorescent Protein (GFP) or its enhanced (EGFP). A non-human mammalian animal comprises an expression system including a nucleic acid encoding said protein.

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## Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

D5 discloses a green fluorescent and luciferase fusion protein. D4 also discloses a method of making monoclonal antibodies to said protein, a method of making the protein and the possibility of its use as a double marker for monitoring gene expression in living cells and quantitatively by enzymatic activity.

D6 discloses primers and probes capable of hybridizing with nucleic acid sequence encoding green fluorescent protein having the length of 14 n.

The Applicant has restricted claims 1 and 14 by indicating the minimum possible length of the protein fragment comprising 10 amino acid residues.

Although an amino acid sequence of the fluorescent protein disclosed in D1 is about 25% identical to the sequence shown in SEQ ID NO:2, it does not comprise fragments having 10 amino acid residues and more, which completely coincide in structure with the claimed protein fragments. Hence, features of claims 1 and 14 are not known from D1 and D2-D6.

Consequently, claims 1-4 and 14 meet the criterion of novelty.

The presence of fluorescent proteins in medusas of the genus *Aequorea* is known from art prior. This information gives grounds for search of similar proteins in other organisms of sea plankton. However, the plankton has a great many of classes, genus, families and phylum of organisms. In this connection it is not obvious for a skilled person the possibility of making a nucleic acid encoding the fluorescent protein and the fluorescent protein from an organism belonging to the phylum Arthropoda.

Hence, an isolated nucleic acid according to claims 1-4 and a protein according to claim 14 and the use thereof for labeling molecules, cells, etc. is not obvious and requires an inventive activity. Based on the foregoing claims 1-4 and 14 meet the criterion of inventive step.

Claims 5-13, 15-24 also meet the criteria of novelty and inventive step, since they contain features of claims 1 or 14.

Claims 1-24 meet the criterion of industrial applicable.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule, which encodes a fluorescent protein, selected from the group consisting of:

5 (a) a nucleic acid which encodes a protein comprising the amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28;

(b) a nucleic acid comprising a nucleotide sequence as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

(c) a nucleic acid that hybridizes under stringent conditions to the nucleic acid of (a) or (b) above;

10 (d) a nucleic acid that encodes a protein that has at least about 60% sequence identity to the amino acid sequence of (a) above;

(e) a nucleic acid that has at least about 55% sequence identity to the nucleotide sequence of (b) above;

15 (f) a nucleic acid which encodes a protein having at least one amino acid substitution, deletion or insertion in the amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.

(g) a derivative or mimetic of the nucleic acid of (a), (b), (c), (d), (e) or (f) above;

(h) a mutant of the nucleic acid of (a), (b), (c), (d), or (e) above;

20 (i) a nucleic acid which differs from the nucleic acid of (b), (c), (d), (e), (f), (g) or (h) above due to the degeneracy of genetic code; and

(j) a fragment of the nucleic acid of (a) or (b) above encoding a peptide of at least 10 amino acid residues in length.

2. The nucleic acid molecule of claim 1, wherein said nucleic acid is isolated from an organism from a phylum *Arthropoda*.

25 3. The nucleic acid molecule of claim 1, wherein said nucleic acid is isolated from an organism from a subclass *Copepoda*

4. The nucleic acid molecule of claim 1, wherein said nucleic acid is isolated from a family *Pontellidae*.

5. A vector comprising the nucleic acid molecule according to claim 1.

30 6. An expression cassette comprising (a) the nucleic acid molecule according to Claim 1; and (b) regulatory elements for the expression of said nucleic acid molecule in the desired host-cell.

7. A cell comprising the nucleic acid molecule according to claim 1, the vector according to claim 5, or the expression cassette according to claim 6.

35 8. A stable cell line comprising the nucleic acid molecule according to claim 1, the vector according to claim 5, or the expression cassette according to claim 6.

9. A transgenic plant comprising the nucleic acid molecule according to claim 1, the vector according to claim 5, or the expression cassette according to claim 6.

10. A transgenic animal comprising the nucleic acid molecule according to claim 1, the vector according to claim 5, or the expression cassette according to claim 6.

5 11. A method for producing a fluorescent protein, said method comprising (a) providing a nucleic acid molecule according to claim 1 operably linked to suitable expression regulatory elements (b) expressing the fluorescent protein from said nucleic acid molecule, and (c) isolating the protein substantially free of other proteins.

12. A nucleic acid molecule comprising a fragment of the nucleic acid molecule  
10 according to claim 1, said fragment encoding a peptide of at least 100 amino acids in length

13. A nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 residues in length of the nucleic acid molecule according to claim 1.

14. An isolated fluorescent protein selected from the group consisting of:

15 (a) a protein comprising the amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8,  
10, 12, 14, 16, 18, 20, 22, 24, 26, or 28;

(b) a protein encoded by the nucleic acid molecule comprising a nucleotide sequence as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

(c) a protein that has at least about 60% sequence identity to the amino acid sequence of  
20 (a) or (b) above;

(d) a mutant of the protein of (a), (b) or (c) above;

(e) a protein having at least one amino acid substitution, deletion or insertion in the amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.

25 (f) a derivative of the protein of (a), (b), (c), (d) or (e) above;

(g) a fragment of the protein of (a), (b), (c), (d), (e) or (f) above comprising of at least 10 amino acid residues in length; and

(h) a protein having a sequence that is substantially the same as, or identical to the amino acid sequence of at least 100 residues in length of (a) or (b) above.

30 15. A fusion protein comprising the protein according to claim 14.

16. An antibody specifically binding to the protein according to claim 14.

17. A kit comprising the nucleic acid according to claim 1, the vector according to claim 5, the expression cassette according to claim 6, the protein according to claim 14, the fusion protein according to claim 15, or a means for producing the same.

35 18. An oligonucleotide probe or primer comprising the nucleotide sequence capable of hybridizing to the nucleotide sequence selected from the group consisting of SEQ ID NOs. 1, 3,

5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27.

19. A method for labeling a biological molecule, comprising coupling said biological molecule to the protein according to claim 14.

5 20. A method for labeling a cell comprising production of the protein according to claim 14 in the cell.

21. A method for labeling a cell organelle comprising production of the protein according to claim 14 fused to the suitable subcellular localization signal in the cell.

22. A method for analyzing a biological molecule, cell or cell organelle comprising detection of fluorescence signal from the protein according to claim 14 or 15.

10 23. A method for analyzing a biological molecule, cell or cell organelle comprising expression of the nucleic acid molecule according to claim 1 in a cell.

24. A method of detecting a biological molecule comprising detection of fluorescence signal from the protein according to claim 14 or 15.